A new hybrid mouse model for agenesis of the corpus callosum

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Abstract

A three locus model of the inheritance of absent corpus callosum in mice was tested by creating F_1 and F_2 hybrid crosses from the strains BALB/cWahl and 129/J which show incomplete penetrance for callosal agenesis. The model predicted that a few or the F_2 hybrid mice would suffer severe reduction of the hippocampal commissure when the corpus callosum was absent, a condition that usually occurs only in the most consistently acallosal I/LnJ strain, and this prediction was confirmed. The $C129F_2$ hybrid population expresses substantial genetic variation and an extremely wide range of defects of the corpus callosum, dorsal commissure of the fornix and hippocampal commissure. At the same time, these hybrids have exceptionally good health and reproductive performance, unlike their inbred parent strains. These characteristics make them ideal subjects for the study of brain—behaviour correlation using a noninvasive method.

Key words: Inbred strain; Incomplete penetrance; Hippocampal commissure; Allometry; Brain—behaviour correlation; Split brain; Genetic analysis; Polygenic

Article:

Introduction

Hereditary absence of the corpus callosum (CC) was first discovered in a stock of mice with rodless retina [12] that became extinct 50 years ago, and the same anatomical defect was later rediscovered in the inbred strains BALB/c, ddN, I/LnJ, and 129, which are not closely related genetically [1,29]. In studies of the genetics, development, and functional consequences of callosal agenesis, each strain has demonstrated advantages as a model, although none appears to be entirely homologous with the human condition [14].

The I/LnJ strain has 100% total absence of the corpus callosum [13] and sometimes suffers severe reduction of the hippocampal commissure (HC) as well [15]. The consistency of the anatomical defect, which appeals to neuroscientists aiming to trace cortical axon pathways, unfortunately presents difficulties to those examining functional consequences of CC absence. Being highly inbred and therefore having many loci randomly fixed for alleles unrelated to the CC defect, the strain may express a variety of spurious behavioural correlates. For example, I/LnJ mice exhibit reduced lateralization of paw preference [10]. However, the strain SWV also is markedly =bilateral [9] but has a perfectly normal CC. Although comparisons of many inbred strains can provide evidence of genetic correlation, crossing studies are required to demonstrate a causal link when phenotypic expression is complete in the inbred strain. Inbreeding also reduces reproductive fitness, and the I/LnJ strain in particular is extremely difficult to procure from the Jackson Laboratory or breed in one's own colony [11].

The BALB/c and 129 strains, which can be obtained readily from commercial sources, both show incomplete penetrance for CC absence in the adult. Strains 129/J and 129/ReJ have 20 to 30% total absence and another 30% with abnormally small CC [14], whereas various sub-strains of BALB/c have from 2 to 20% total CC absence [27]. In both or these strains the HC is almost always normal in the adult, although its formation is markedly delayed in the embryo [5,26]. Because phenotypic variation within the BALB/c strain is not hereditary [27], it presents a seemingly ideal experiment of nature for examining developmental and functional

consequences of CC absence; genetically identical littermates sharing a highly similar environment from conception onwards have radically different callosal anatomies. Incomplete penetrance has been used to advantage in tract tracing studies to document reduced density but normal topographical patterns of transcortical connections when the CC is very small [17]. To date the only behaviour thoroughly examined in these strains in relation to CC size is lateralized paw preference. Two studies with relatively large samples found no significant association between degree of paw preference and cross-sectional area of the CC at the midsagittal plane in BALB/c mice [6,22]. Because an inbred strain represents only one genotype, results lack generality to other strains. Indeed, there is evidence of reduced laterality when the CC is small or absent in the 129/J strain [30].

The ddN strain, maintained only in Japan, has relatively low (4 to 8%) frequency of total CC absence [18], and excellent anatomical studies of cortical axon pathways have required heroic sample sizes to obtain enough abnormal animals [19]. Mice of this strain lacking the CC exhibit reduced correlations of EEG activity both between and within hemispheres [8,16].

Lipp and coworkers devised an improved mouse model of callosal agenesis by crossing I/LnJ to the normal strain C57BL/6J and then backcrossing to I/LnJ for several generations [13]. In the second generation backcross a few mice with absent CC were obtained and by the fourth backcross almost all suffered total absence of the CC. Absent CC appeared in hybrid mice that were vigorous and reproduced well, and these animals proved useful for research on motor coordination deficits [14]. This approach suffers from two drawbacks, however. The I/LnJ for backcrossing are very difficult to procure or breed, and prolonged backcrossing inevitably leads to a sharp decline in fertility.

Recent discoveries in our laboratory have resulted in an improved hybrid mouse model which is easily bred from commercially available inbred strains and is exceptionally vigorous. Furthermore, it expresses an extreme range of CC and HC sizes, making it an excellent subject for research on functional consequences of callosal agenesis. Although the BALB/cWahl and 129/ReJ inbred strains both show a moderately high frequency of callosal deficits, their F_1 hybrid is normal [15], which indicates the strains differ at two or more loci relevant to CC agenesis. F_1 hybrids among the other strains yield CC deficits similar to the parent strains or midway between them [15]. These and other data support a three locus model of CC agenesis where a mouse must be homozygous recessive at two or more loci to suffer a CC deficit and homozygosity at three loci causes a more severe syndrome than homozygosity at two loci (see Table 1). This model predicts that the BALB/c x 129 F_1 hybrid cross will have genotype *b/ + + /c* and therefore be normal. To test this model F_2 hybrid can be formed by crossing F_1 hybrids. consequences of such a cross (Table 2) should include 1/16 of the mice homozygous at all three loci and therefore phenotypically similar to I/LnJ, which is the strain with a high frequency of deficient FTC [15]. Because not all I/LnJ mice have severely reduced HC, it is expected that the F_2 hybrid group will have a little less than 6% of mice with reduced HC.

Table 1
Three locus model of inheritance of corpus callosum deficiency sh
substrain-specific alleles

Strain	*A* Locus	*B* Locus	*C* l
C57BL/6J	+ / +	+ / +	+ /
BALB/cWahl	a^W/a^W	b^W/b^W	+ /
129/ReJ	a^R/a^R	+ / +	$c^R/$
I/LnJ	a^L/a^L	b^L/b^L	$c^L/$

Table 2
Prediction* from the three locus model in Table 1 for the F_2 hybrid cro
of BALB/cWahl and 129/ReJ

Frequency	Genotypes	Brain stat
9/16	+ / − + / −	Normal
3/16	b^W/b^W + / −	Like BAL
3/16	+ / − c^R/c^R	Like 129
1/16	b^W/b^W c^R/c^R	Like I/Ln

* Only results for loci *B* and *C* are shown because the two strains a both homozygous recessive at locus *A*. The symbol + / − indicates genotype with at least one dominant, wild-type allele; the other alle could be + or recessive.

2. Materials and methods

2.1. Animals

A normal F_2 hybrid comparison group (termed B6D2F2) was obtained by crossing B6D2F_1/J mice (cr…of a

C57BL/6J female by a DBA/2J male) purchased from the Jackson Laboratory, Bar Harbor, ME (USA). This hybrid has a wide range of genetic variation because the parent strains are so different genetically [1,24], but it never exhibits defects of the CC or HC. The BALB/cWa strain is maintained in the authors' laboratory [2' whereas the 129/J strain was obtained from the Jacks Labs. A BALB/cWahl female was crossed with a 129/J male to obtain C129F$_1$ mice, which were then crossed obtain F$_2$ hybrids (termed C129F2).

2.2. Breeding

One male was placed in a standard (29 × 18 × 13 cm) plastic mouse cage with one to three females and the female was isolated in a clean cage when visibly pregnant. At mating and throughout pregnancy and lactation the parent mice were fed freely with high fat Wayne Mouse Breeder Blox 8626 and given free access to Edmonton tap water. The cage contained Aspen Chip bedding and one compacted paper "Nestlet" for nest construction. The pups were weaned and housed in groups of same-sex littermates at 20 to 22 days after birth. For the next week they received the standard, low-fat diet (Wayne Rodent Blox 8604).

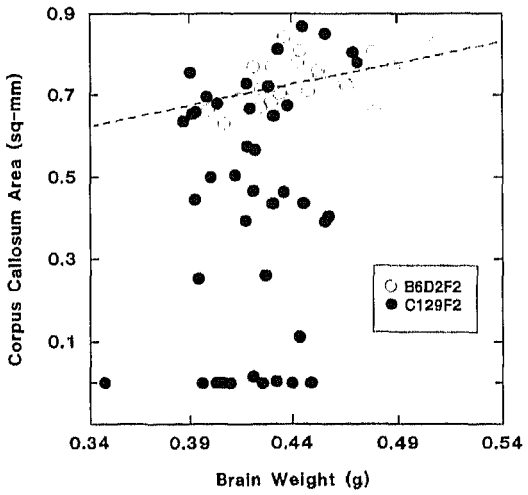

Fig. 1. Cross-sectional area of the corpus callosum at the midsagittal plane (mm^2) versus brain weight (g) for hybrid mice of the B6D2F2 (open circles) and C129F2 (black dots) groups. The dashed line is the regression line of best fit for the normal B6D2F2 hybrid group.

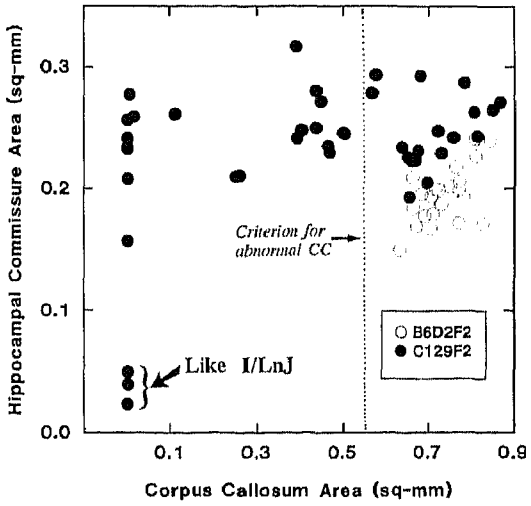

Fig. 2. Cross-sectional area of the hippocampal commissure at the midsagittal plane (mm^2) vs. area of the corpus callosum (mm^2) for mice of two F$_2$ hybrid crosses. Note that all of the B6D2F2 and many of the C129F2 animals are above the criterion for a normal corpus callosum, 0.55 mm^2, indicated by the dotted line. Of greatest importance is the occurrence of three C129F2 mice with reduced hippocampal commissure, which is usually seen only in the acallosal strain I/LnJ.

2.3. Histology

At 27 to 29 days after birth, each mouse was anesthetized with a pentobarbital overdose (120 mg/kg) and perfused intracardially with 10 to 15 ml of vascular rinse followed by 25 to 30 ml of neutral 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were extracted from the skull and placed in fresh fixative. One week later they were trimmed to a standard configuration [25], blotted, and weighed. Frozen sagittal sections were then cut at 30 pm and stained with gold chloride for myelin [23].

2.4. Measurements and analysis

The section of good quality closest to the midsagittal plane was traced with a Leitz tracing device at 40 × to reveal the outlines of the corpus callosum (CC) proper, the dorsal commissure of the fornix (DCF), the hippocampal commissure (HC), and the anterior commissure (AC). Care was taken not to include either the longitudinal striae or the superior fornix in the tracing of the CC. The AC, HC, and CC were traced from different sections if mid-plane varied because of small errors in the angle of slicing. The cross-sectional areas of the CC, HC, and AC were measured with a digitizing tablet and the Sigma Scan program from Jandel Scientific, and statistical analysis was done with the multiple regression program of SYSTAT. Because several statistical tests were done, the probability of a Type I error was set at $\alpha = 0.01$. Almost all tests were directional.

3. Results

Good data were obtained from three litters of B6D2F2/J containing 23 mice (litter sizes 2, 9, 12) and four litters

of C129F2 with 43 mice (litter sizes 10, 11, 11, 11). The reproductive performance of the C129F1 mice was outstanding , as were the health and vigour of their offspring.

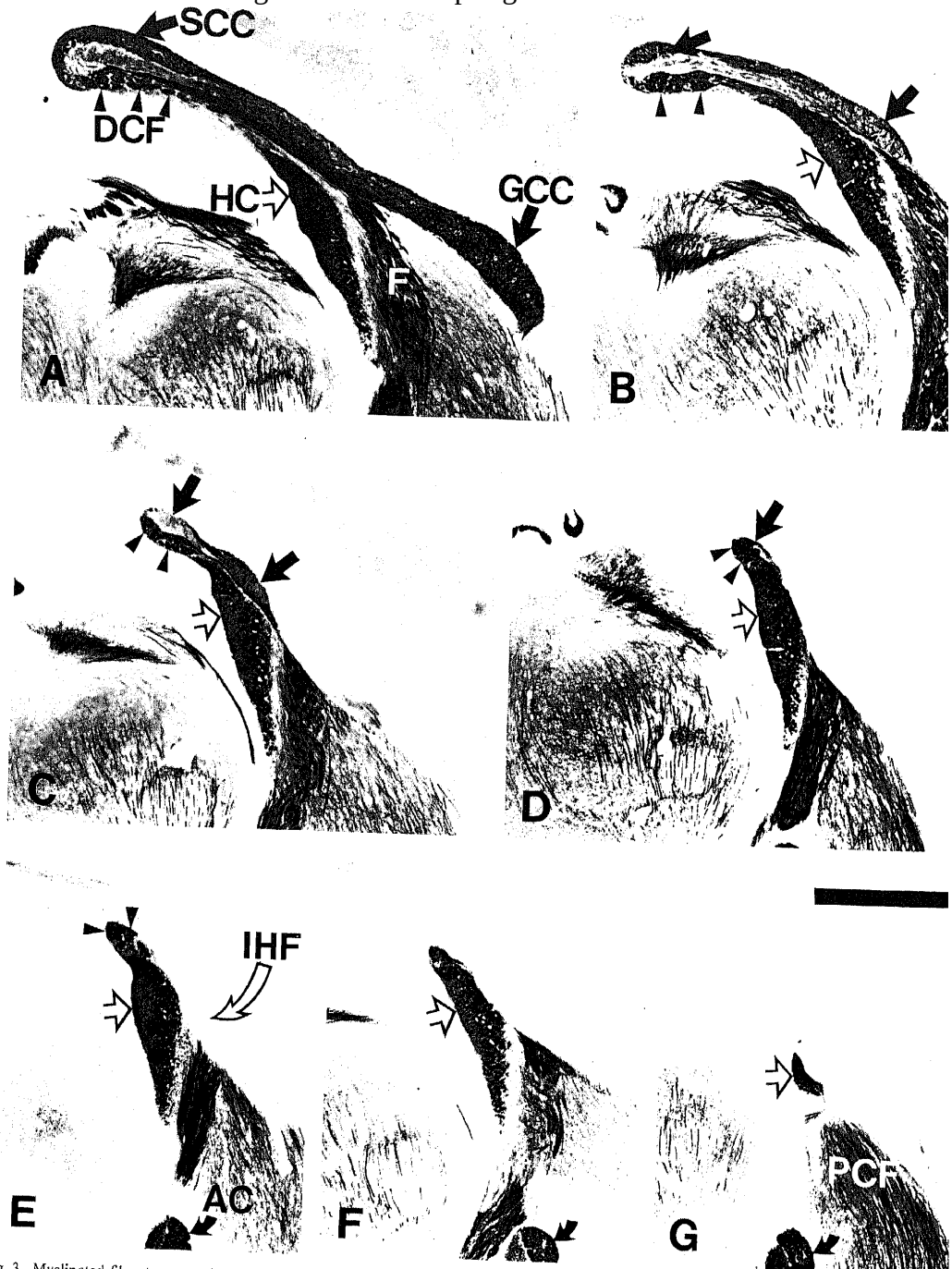

Fig. 3. Myelinated fibre tracts at the midsagittal plane for several mice of the C129F2 hybrid cross. The corpus callosum (CC) is shown by a lar arrow, the dorsal commissure of the fornix (DCF) by small arrowheads, the hippocampal commissure (HC) by an open arrow, and the anteri commissure (AC) by a curved arrow. A: example of a normal mouse. B: an animal with reduced CC area that does not exhibit the typically bulbo splenium of the corpus callosum (SCC) or thick genu of the corpus callosum (GCC). C: the CC is very small and directly above the HC. It wou be unwise to label the portions of the corpus callosum SCC and GCC without the aid of tract tracing to determine cortical regions of origin. The DCF and HC are normal, although the DCF is closer to the HC than in A. D: there is only a tiny fragment of the CC present, but the DCF and HC have normal sizes. The DCF is now directly above the HC. E: no CC is present and the DCF is above the HC. F: no CC or DCF is present. G: even the HC is greatly reduced. The fornix ("F") is displaced away from midplane because of a bulge in the interhemispheric fissure (IHF), although the precommissural fornix (PCF) is present. Scale bar = 1.0 mm.

3.1. Body and brain sizes

Males (17.9 g) were significantly heavier than females (15.8 g) in a litter means analysis (t_5 = 4.4, P= 0.004), but the two hybrid crosses had similar body sizes. On the other hand, there was no significant sex effect or interaction with sex for brain weight, and brain weight was best described by the equation BRAIN(g)= 0.322 + 0.0066 × BODY — 0.0219 × GROUP with adjusted multiple R^2 = 0.404. When equated for body weight, C129F2 mice had brains about 21.9 mg smaller than B6D2F2/J mice (P= 0.0003).

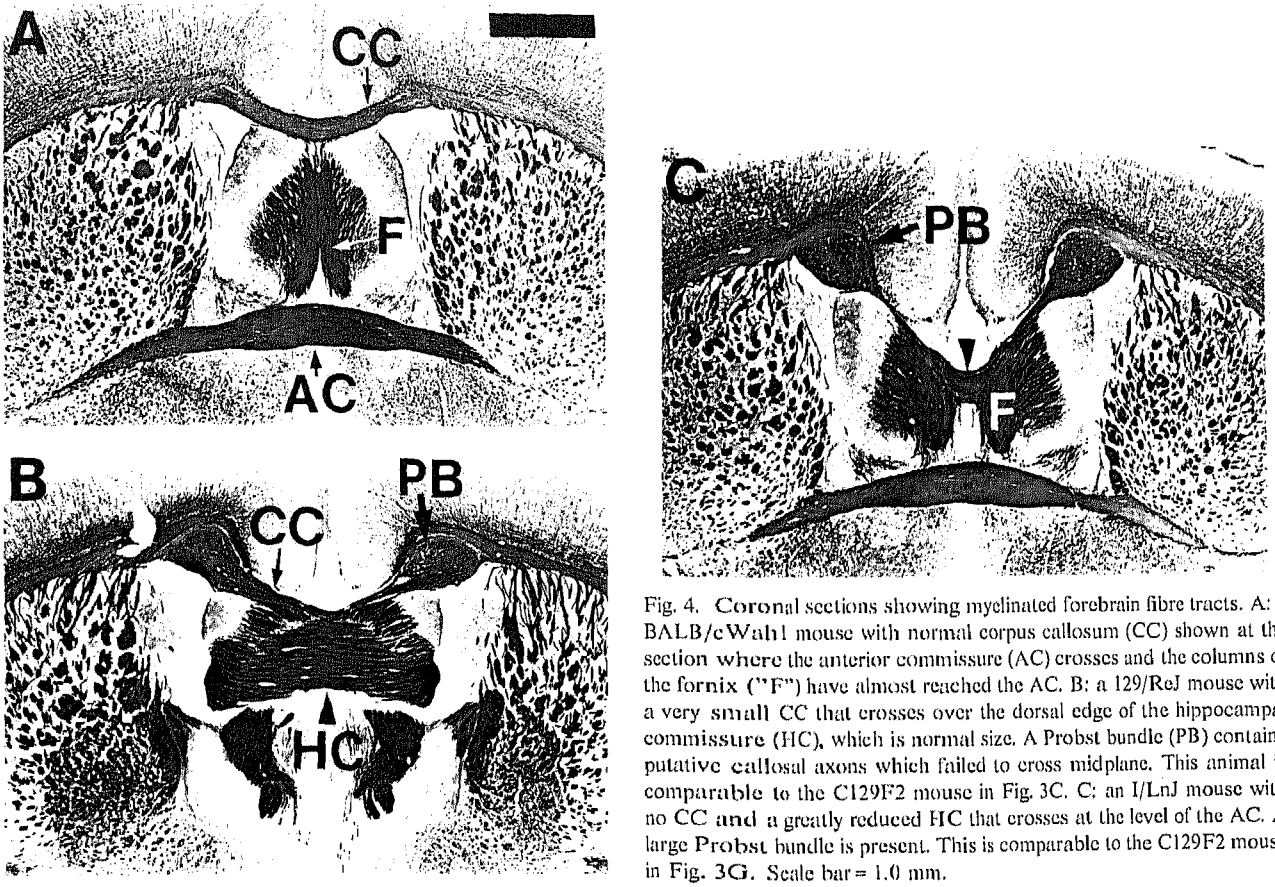

Fig. 4. Coronal sections showing myelinated forebrain fibre tracts. A: a BALB/cWah1 mouse with normal corpus callosum (CC) shown at the section where the anterior commissure (AC) crosses and the columns of the fornix ("F") have almost reached the AC. B: a 129/ReJ mouse with a very small CC that crosses over the dorsal edge of the hippocampal commissure (HC), which is normal size. A Probst bundle (PB) contains putative callosal axons which failed to cross midplane. This animal is comparable to the C129F2 mouse in Fig. 3C. C: an I/LnJ mouse with no CC and a greatly reduced HC that crosses at the level of the AC. A large Probst bundle is present. This is comparable to the C129F2 mouse in Fig. 3G. Scale bar = 1.0 mm.

3.2. Corpus callosum

There was no significant sex or sex interaction effect on CC size ($P > 0.5$). but C129F2 mice had smaller and much more variable CC size than B6D2F2. As shown in Fig. 1, among the normal B6D2F2 hybrid mice the CC was larger in those with larger brains (CC = 0.271 + 1.043 × BRAIN, $r = 0.55$, $P = 0.004$), whereas the CC was not significantly related to brain size in C129F2 ($r = 0.22$, $P = 0.08$). As noted previously [4] with BALB/c, for those C129F2 mice with a CC in the normal size range there was a positive association with brain size and this allometric relation was very similar to that of the normal B6D2F2 hybrids (Fig. 1). If the criterion for abnormally small CC is set at 0.55 min^2, appropriate for 28 days and precision tracing, there were 25 of 43 or 58% of C129F2 mice with deficient CC. Among these, 10 mice or 23% had no CC axons crossing mid-plane, which is reasonably close to the expectation of about 17% from the model in Table 2.

3.3. Hippocampal commissure

Plotting HC area against CC area (Fig. 2), it is apparent that there were no deficits in the I-IC in C129F2 mice which had at least a few CC axons crossing midplane. Among those with no CC axons crossing midplane, three had an unquestionably small HC of less than 0.05 mm^2, much like the I/LnJ strain. The fraction 3/43 = 7% is somewhat more than the maximum of 6% or 1/16 expected from the model in Table 2. The one C129F2 mouse with HC area of 0.156 mm^2 was near the borderline for abnormality. Because its brain weight (0.403 g) was very close to the 0.407 g of a B6D2F2 mouse having an HC area of 0.149 mm^2, it should not be counted as a case of deficient HC. The strain C57BL/6J tends to have a relatively small HC and so did the B6D2F2 hybrids (Fig. 2). It is reasonable to draw the line for abnormality below the limit for mice from a normal hybrid comparison group.

Fig. 3 shows examples of C129F2 mice with a wide range of CC and HC sizes. The normal brain (Fig. 3A) is indistinguishable from a B6D2F2 mouse with a similar brain size. When far fewer CC axons cross midplane, they are invariably centered over the HC (Fig. 3B,C), which in the embryo can act as a bridge for CC axons across the interhemispheric fissure [20,21,27]. Although the typically bulbous splenium of the CC is often not

apparent when the CC is very small, tract tracing studies indicate that axons from occipital cortex are usually present in a very small CC [17,21]. In rare instances (Fig. 3D), a tiny patch of CC axons is located directly apposed to the HC, whereas the bulk of the putative CC axons course ipsilaterally in the Probst bundle (Fig. 4B). The dorsal commissure of the fornix (DCF) is always present when any amount of CC axons crosses midplane, and the DCF is sometimes present when the CC is totally absent. If the DCF is present, the HC always has normal size (Fig. 3E), but the HC can be nearly normal size without having any DCF above it (Fig. 3F). Finally, the HC itself can be greatly reduced (Fig. 3G). Thus, there is a continuum of rnidline commissure defects, and their severities correspond to the degree of retarded formation of the HC in the embryo. The range from normal CC, to very small CC with normal HC, to very small HC is shown in coronal sections in Fig. 4.

3.4. Anterior commissure

The AC was larger in mice with bigger brains (AC = 0.007 + 0.249 × BRAIN, adj. R^2 = 0.42, P<0.00001) but adding dummy variables for sex, hybrid group or interaction did not increase the multiple R^2 significantly ($F_{3,61}$ = 1.04, P= 0.38). As shown in Fig. 5, the AC of C129F2 mice tended to be smaller than B6D2F2 mice, as did brain size, but the relation between AC and brain size was very similar for the two groups. Inspection of other commissures in the diencephalon and mesencephalon revealed no apparent abnormalities in these mice. The problem in C129F2 mice seems to be restricted to the dorsal septal region where the HC and CC cross midplane in the embryo [28].

4. Discussion

The F_2 hybrid cross of BALB/cWahl and 129/ReJ inbred strains yields about as many mice with total absence of the corpus callosum (23%) as either parent strain and it also provides a few animals with greatly reduced hippocampal commissure, a defect seen with moderate frequency only in the I/LnJ strain. These results are consistent with the three locus genetic model presented in Table 1, although additional evidence from a larger replicated sample and genetic linkage with marker loci will be required to prove this conclusively. A possibility remains that a polygenic model with two thresholds [3], one for deficient CC and another deficient HC, could account for the present results. Whether three or many loci combined to prevent formation of the corpus callosum, the extreme range of phenotypes in the F_2 hybrid cross will confer high statistical power on tests of brain-behaviour correlation.

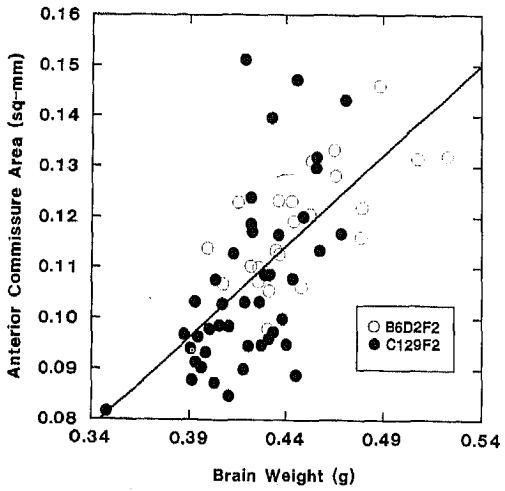

Fig. 5. Cross-sectional area of the anterior commmissure (mm^2) at the midsagittal plane vs. brain weight (g) for the two F_2 hybrid groups, showing the line of best fit for the two groups combined.

Unlike the parental inbred strains, the F_2 hybrid mice are highly fertile, healthy and vigorous behaviourally, which should make them ideal subjects for research on the functional consequences of callosal agenesis. Because there is great genetic variation among the hybrids, results will have greater generality than with extreme inbreds, which are atypical mice created by forced inbreeding. The F_2 genetic variance attenuates the problem of spurious correlation of behaviours with CC deficiency. Closely linked loci would remain associated with genes producing CC agenesis in the F_2, but there is no reason to expect that alleles yielding a particular deviation from the group 01000 for a behaviour would consistently be paired with alleles at nearby loci leading

to CC defects. In any event, all hut the closest linkage could be ruled out by further generations of random mating.

This new hybrid mouse model allows investigation of the roles not only of the corpus callosum but also the dorsal commissure of the fornix and the hippocampal commissure. A sample of at least 100 mice would be necessary to obtain sufficient animals in the less common categories of absent or reduced commissures. Fortunately, this could easily be achieved with only four or five $C129F_1$ breeding females and two males, given their outstanding reproduction. This technique is probably the only effective way to obtain a mouse with intact DCF and HC but no CC, because section of the adult CC almost certainly will sever the DCF. Hereditary agenesis of a structure can achieve a degree of precision and freedom from surgical artifacts that are impossible with surgery. Of course, there may be developmental compensation for failure of a structure to form which leads to a different pattern of functional impairment compared with surgical section in the adult. The destinations of axons which fail to cross in the dorsal or ventral commissures of the fornix are unknown at present. It will be interesting to study the altered topography of commissural connections in the hippocampus in relation to spatiotemporal gradients [7] altered by the hereditary midline defect.

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